



# Image Processing and Filtering Robert Haase

With material from

Marcelo Leomil Zoccoler and Till Korten, PoL TU Dresden

Mauricio Rocha Martins, Norden lab, MPI CBG

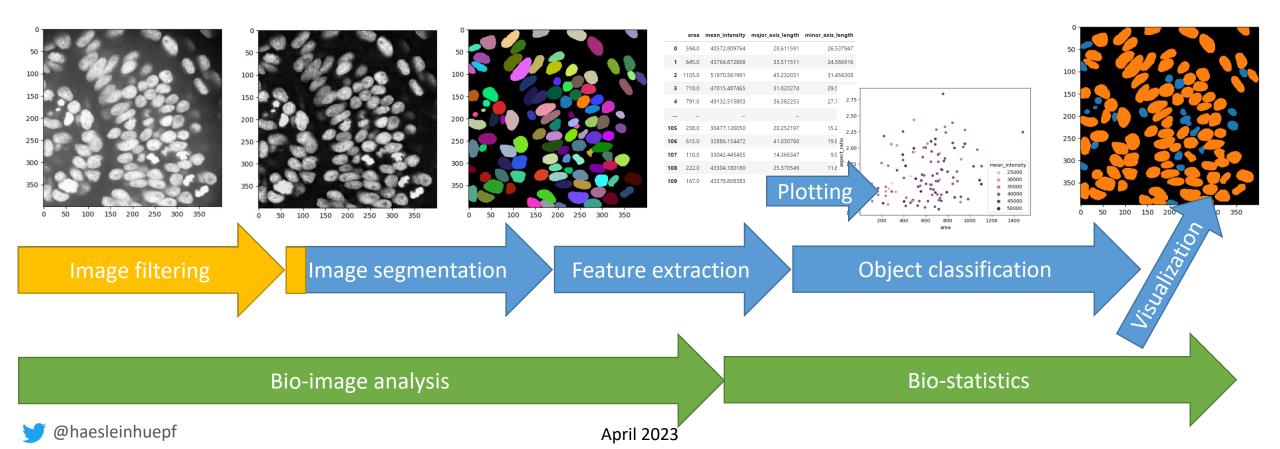
Dominic Waithe, Oxford University

Alex Bird, Dan White, MPI CBG



#### Lecture overview: Bio-image Analysis

- Image Data Analysis workflows
- Goal: Quantify observations, substantiate conclusions with numbers



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# Image Visualization

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- An image is just a matrix of numbers
- Pixel: "picture element"
- The edges between pixels are an artefact of the imaging / digitization. They are not real!

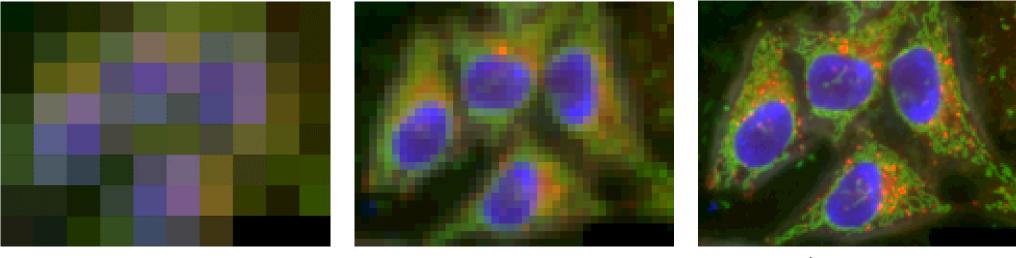
48	48	48	40	40	32	32	24	24	24	24	24	24	24
48	48	40	32	32	24	24	16	16	16	24	24	24	24
48	48	40	32	24	24	16	16	16	16	24	24	32	40
40	40	32	24	24	16	16	8	16	16	24	24	40	48
32	32	32	24	24	16	24	24	32	48	56	64	72	88
24	24	24	16	16	16	24	32	56	72	88	96	112	120
24	16	16	16	24	32	48	64	96	120	128	144	152	152
16	8	16	16	32	40	72	96	128	160	176	184	184	184
16	8	16	24	48	72	104	136	160	176	184	192	192	184
16	8	24	32	72	104	136	168	184	192	200	200	192	184
24	24	48	64	104	136	160	184	184	192	192	192	184	184
32	40	64	88	128	168	184	192	192	184	184	176	176	176
40	56	88	120	152	192	192	192	192	184	184	176	176	176
48	64	104	144	176	208	200	184	184	184	184	176	176	168



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#### Pixel size versus resolution

- Pixel size is a digital property of an image.
- You configure it during the imaging session at the microscope.



Pixel size:  $3.3 \ \mu m$ 

Pixel size: 0.8  $\mu m$ 

Pixel size: 0.05 µm

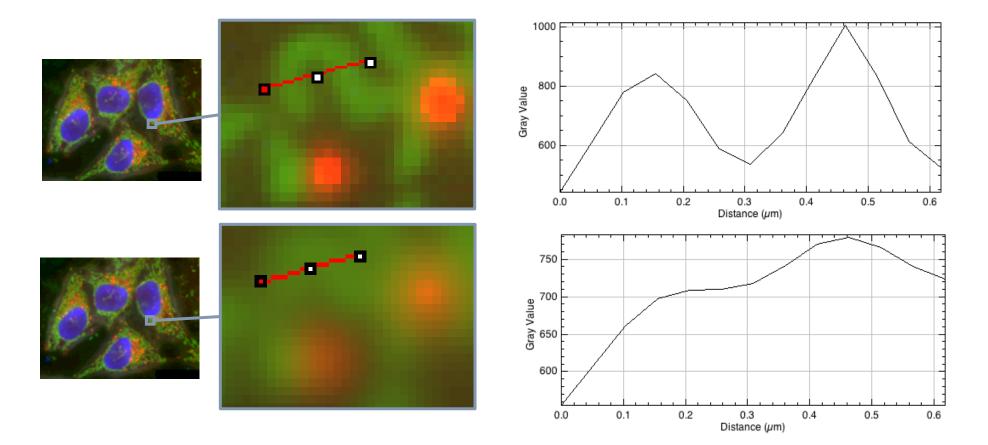
Po

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• We are not talking about resolution!



- Resolution is a property of your imaging system.
- The measure of how close object can be in an image while still being differentiable, is called spatial resolution.



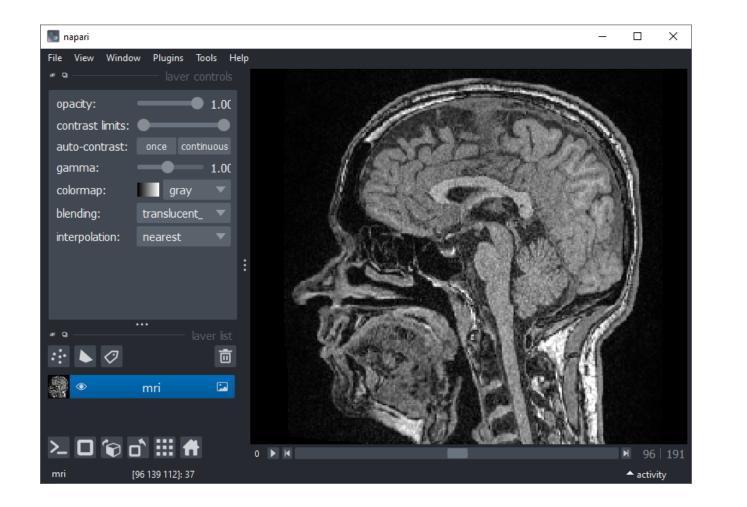
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#### Image stacks and voxels



#### There are tools available for exploring them





#### https://github.com/haesleinhuepf/stackview

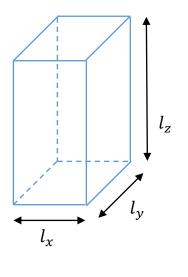


#### <u>https://napari.org/</u>

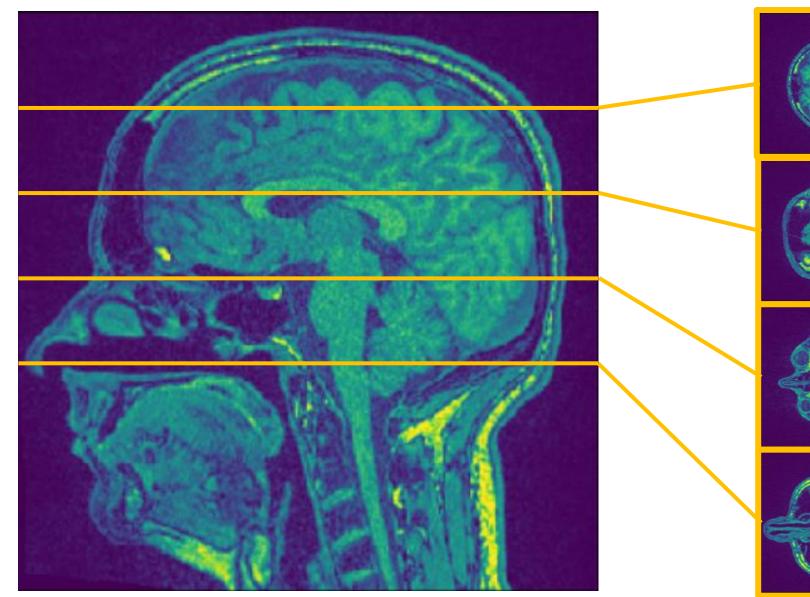
#### Image stacks and voxels

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- 3-dimensional images consisting of voxels
- "Image stack"
- Often anisotropic (not equally large in all directions)



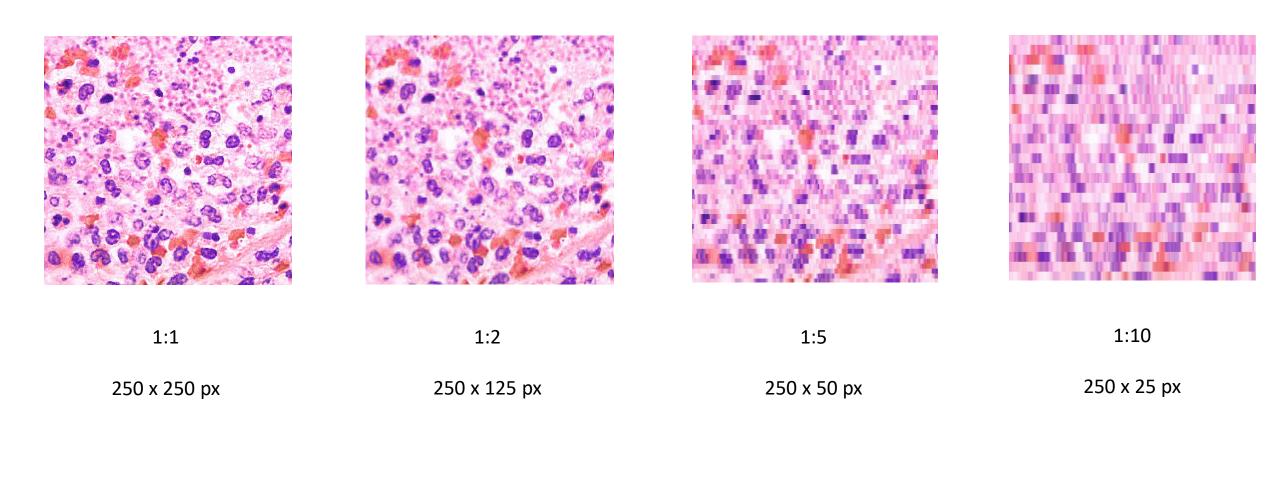
 $l_x = l_y \neq l_z$ 



Øhaesleinhuepf



• Voxel size has immediate impact on image quality and thus, on processing / analysis results.



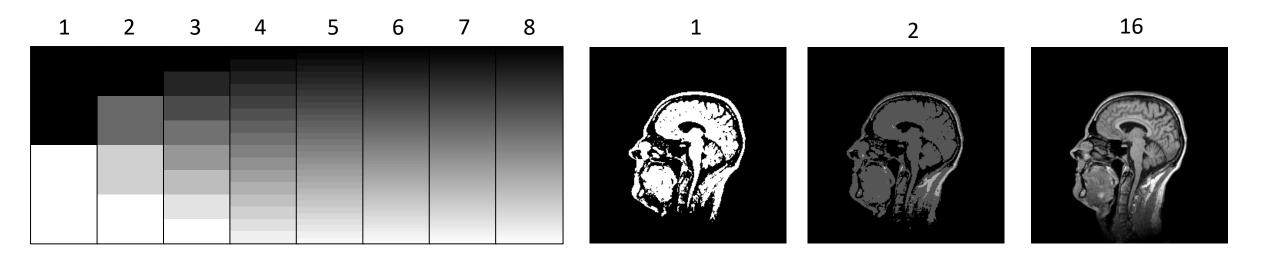
🈏 @haesleinhuepf

April 2023

Image source: cropped from <a href="https://de.m.wikipedia.org/wiki/Datei:Histo\_Lungenpest.jpg">https://de.m.wikipedia.org/wiki/Datei:Histo\_Lungenpest.jpg</a>

#### Bit-depth

- A bits is the smallest memory unit in computers, atomic data.
- The bit-depth *n* enumerates how many different intensity values are present in an image:
  - 2<sup>n</sup> grey values
- In microscopy, images are usually stored as 8, 12 or 16-bit images.



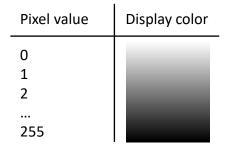
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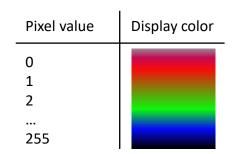


# Colormaps / lookup tables

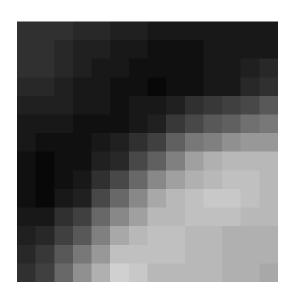
- The lookup table decides how the image is displayed on screen.
- Applying a different lookup table does not change the image. All pixel values stay the same, they just appear differently

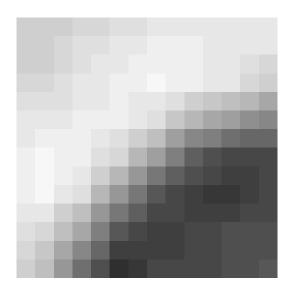
Pixel value	Display color
0 1 2	
 255	

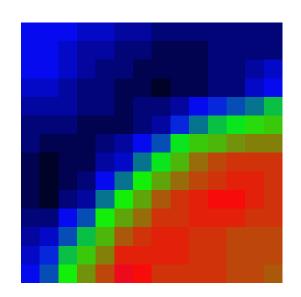




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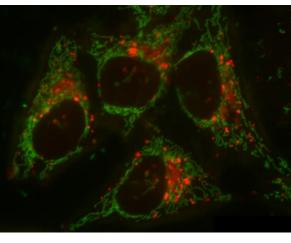




### Colormaps / lookup tables

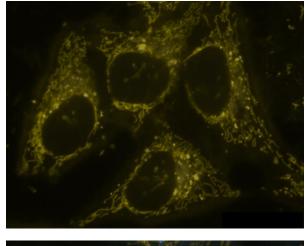


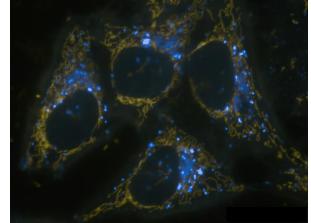
- Choose visualization of your color tables wisely!
- Think of people with red/green blindness!



#### Common view





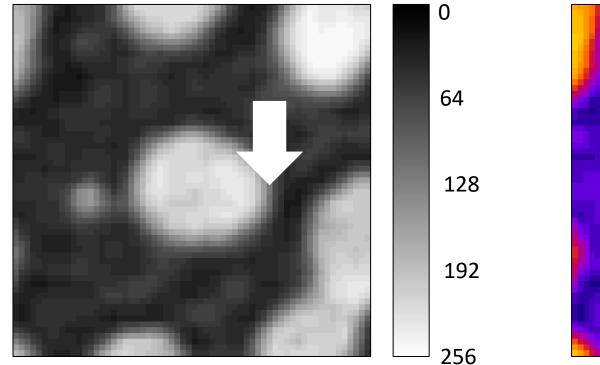


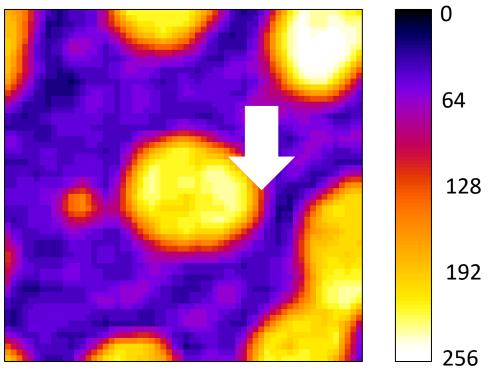
Replace red with magenta!

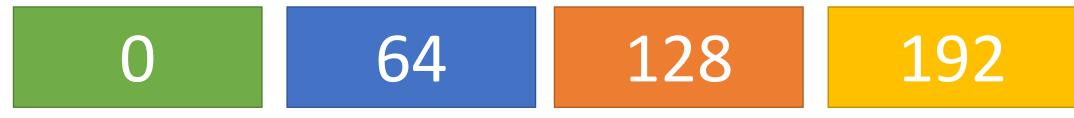
@haesleinhuepf



#### • Which intensity does the marked pixel have?



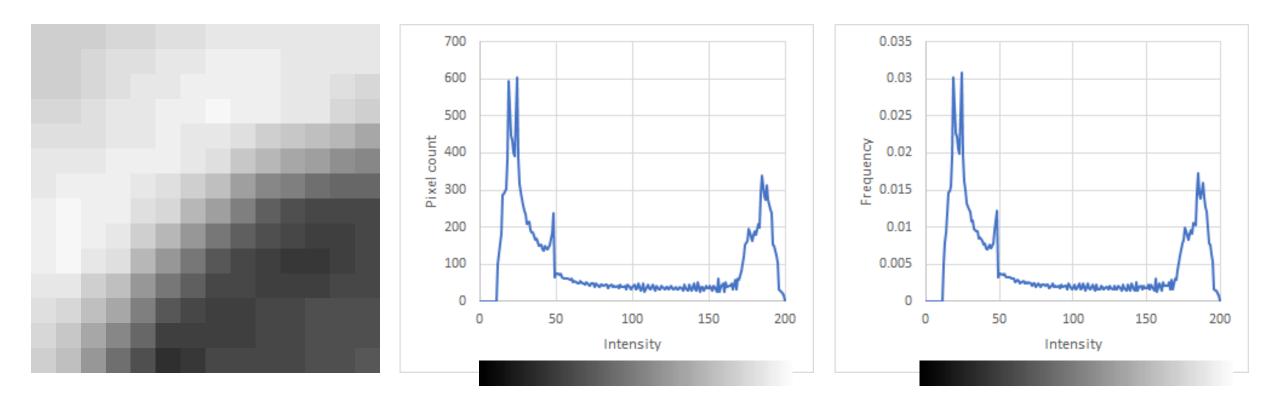








- A histogram shows the probability distribution of pixel intensities.
- The probability of a pixel having a certain grey value can be measured by counting pixels and calculating the frequency of the given intensity.
- Whenever you see a histogram, try to imagine the lookup-table on the X-axis

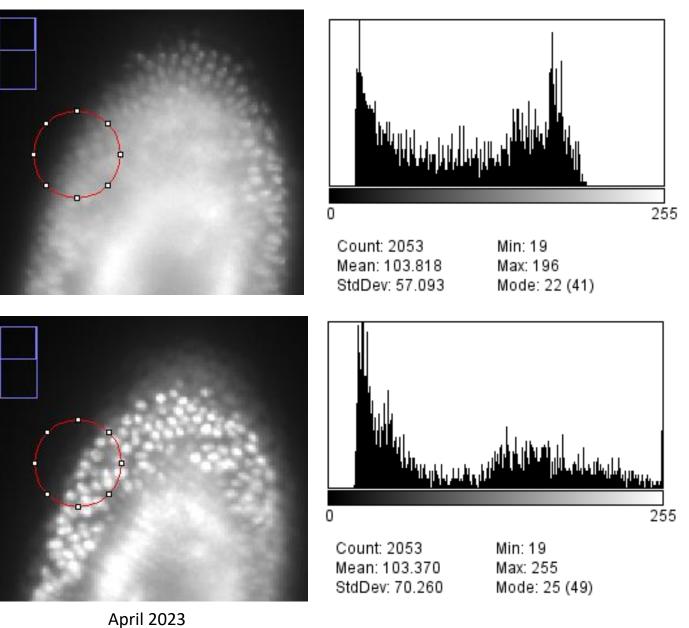




#### Histograms



- Histograms are summaries of images
- Tell stories, e.g. about image quality

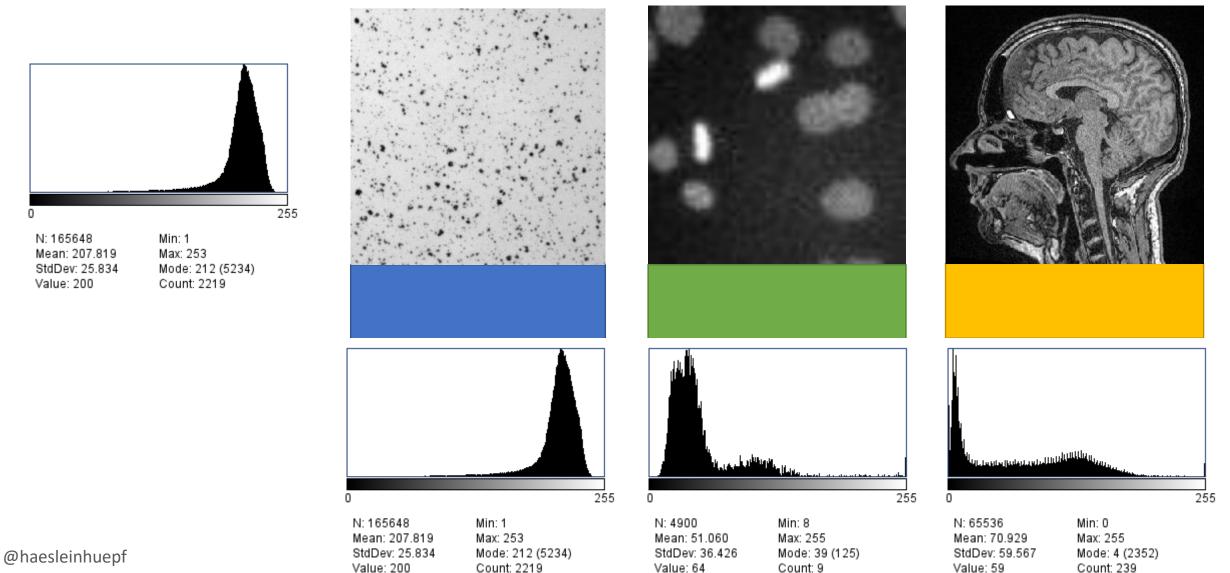




#### Histograms



• To which of the three images does this histogram belong to?







# Image Filtering

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With material from

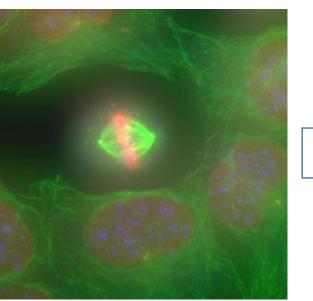
Marcelo Leomil Zoccoler and Till Korten, PoL, TU Dresden



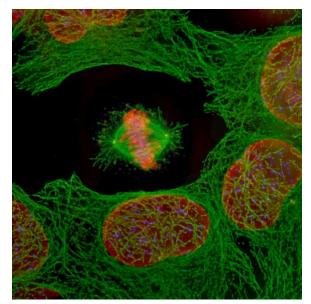




- An image processing filter is an operation on an image.
- It takes an image and produces a new image out of it.
- Filters change pixel values.
- There is no "best" filter. Which filter fits your needs, depends on the context.
- Filters do not do magic. They can not make things visible which are not in the image.
- Application examples
  - Noise-reduction
  - Artefact-removal
  - Contrast enhancement
  - Correct uneven illumination



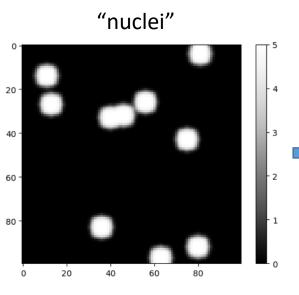
Filter



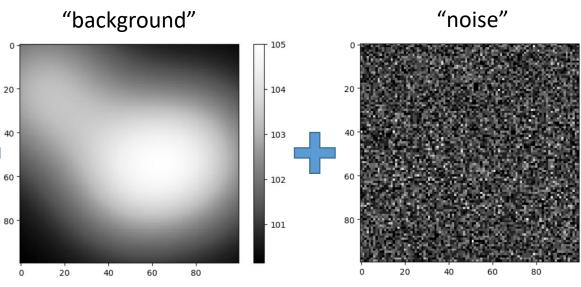




• Image formation (simulated)



- Aberrations, defocus
- Motion blur

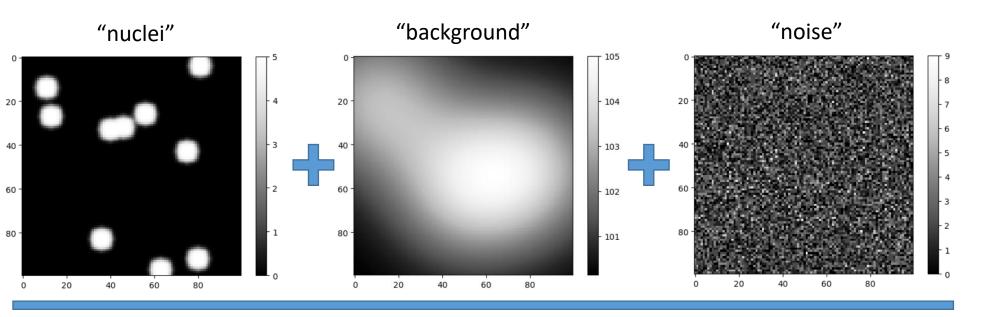


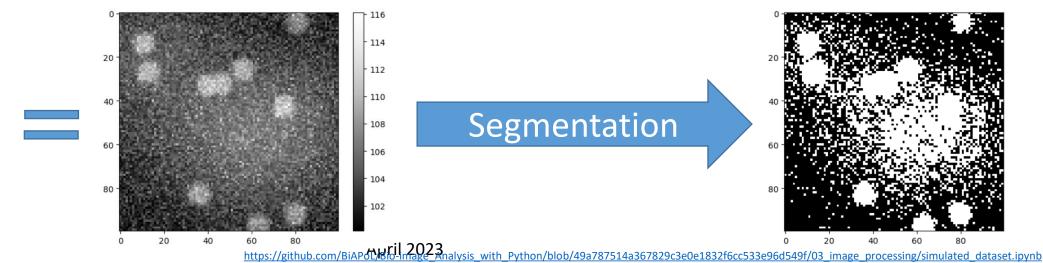
- Light from objects behind and in front of the scene (out-of-focus light)
- Dirt on the object slide
- Camera offset

- Shot noise (arriving photons)
- Dark noise (electrons made from photons)
- Read-out-noise (electronics)



• Image formation (simulated)



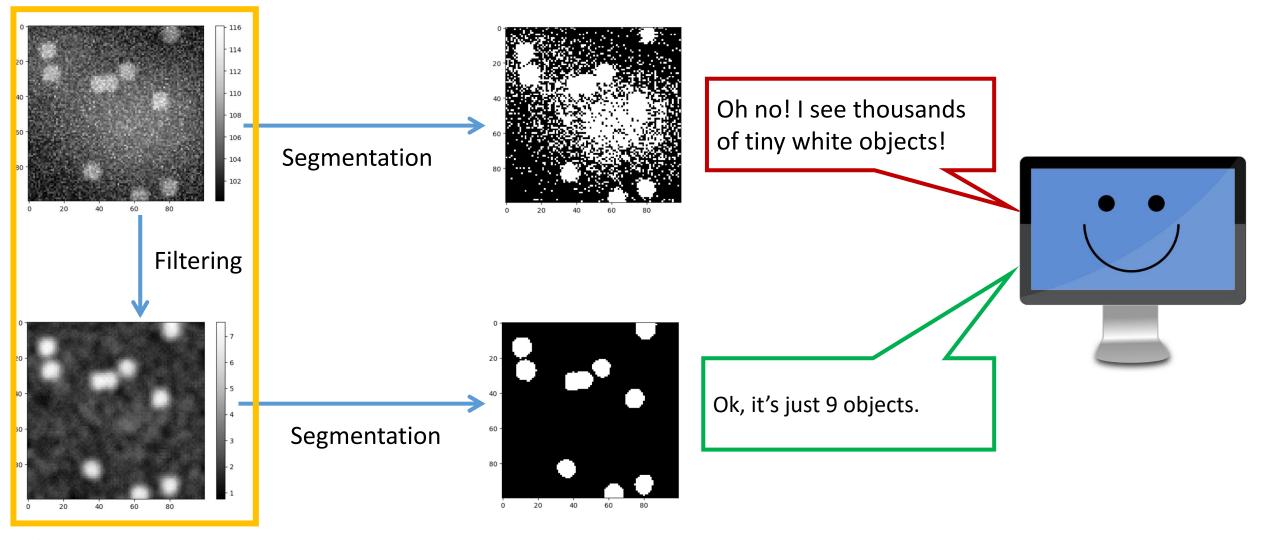




## Image filtering



• We need to remove the noise to help the computer *interpreting* the image

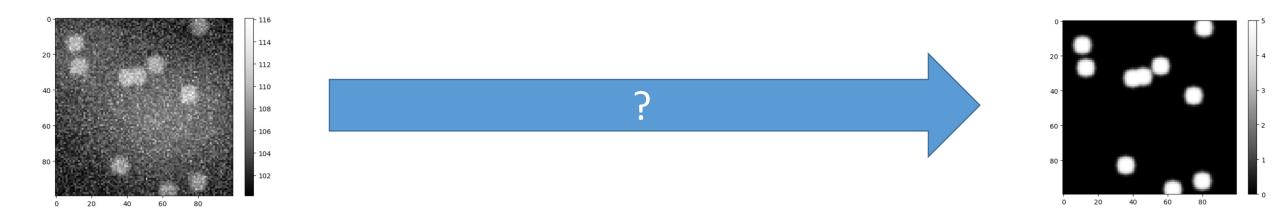


) @haesleinhuepf

## Image filtering



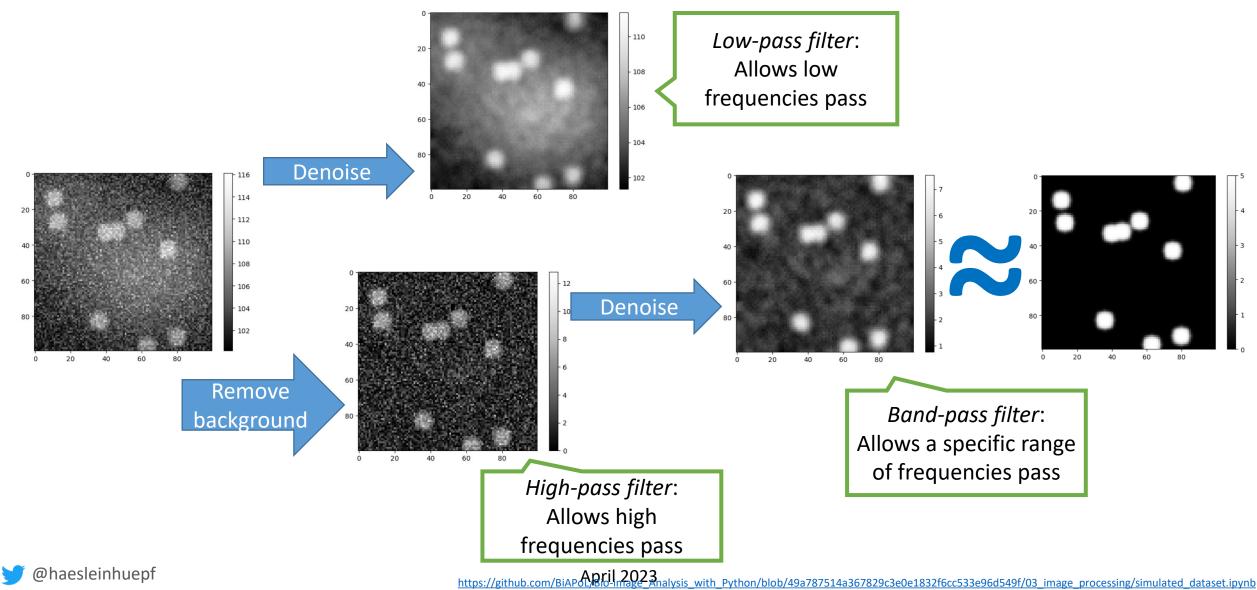
• Attempt to invert / "undo" processes disturbing image quality



# Image filtering

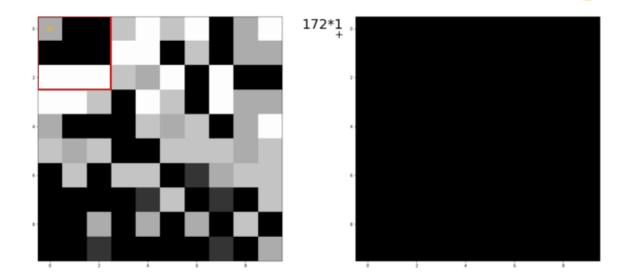


• Attempt to invert / "undo" processes disturbing image quality



#### Linear Filters

- *Linear filters* replace each pixel value with a weighted linear combination of surrounding pixels
- Filter *kernels* are matrices describing a linear filter
- This multiplication of surrounding pixels according to a matrix is called *convolution*



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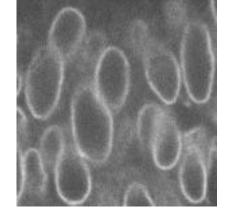
	1/9	1/9	1/9	
Mean filter, 3x3 kernel	1/9	1/9	1/9	
	1/9	1/9	1/9	

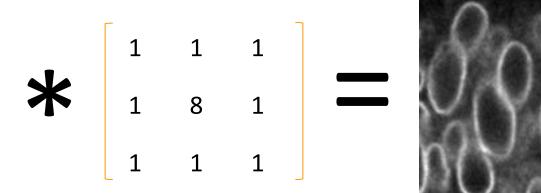


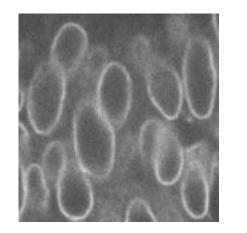
# Linear filters

- Terminology:
  - "We convolve an image with a kernel."
  - Convolution operator: \*

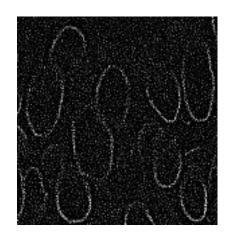
- Examples
  - Mean
  - Gaussian blur
  - Sobel-operator
  - Laplace-filter







0 0 -1 4 -1 0

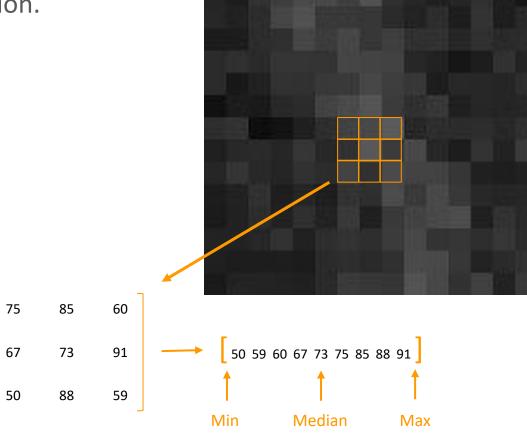


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- Non linear filters also replace pixel value inside as rolling window but using a non-linear function.
- Examples: order statistics filters
  - Min
  - Median
  - Max
  - Variance
  - Standard deviation

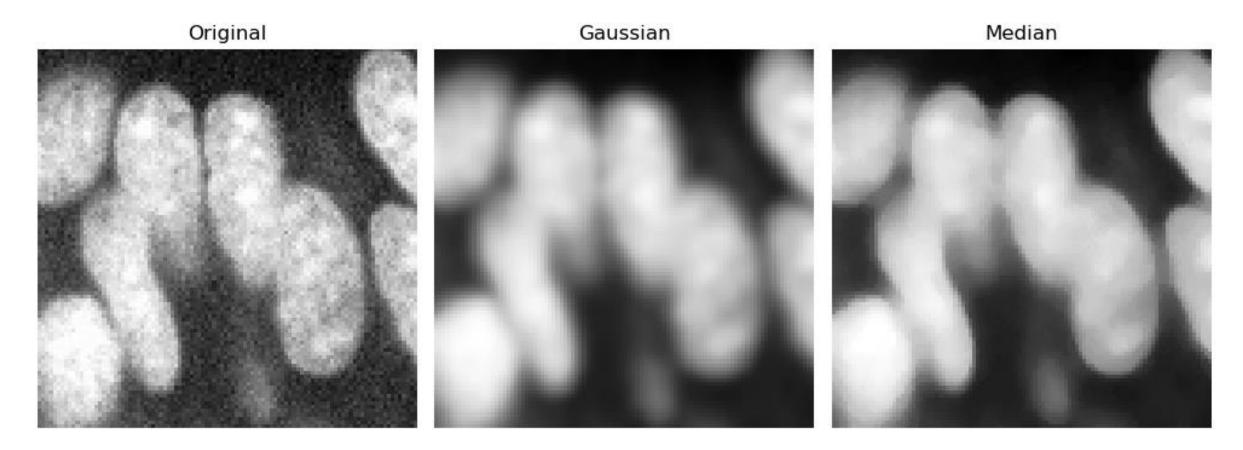


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#### Noise removal

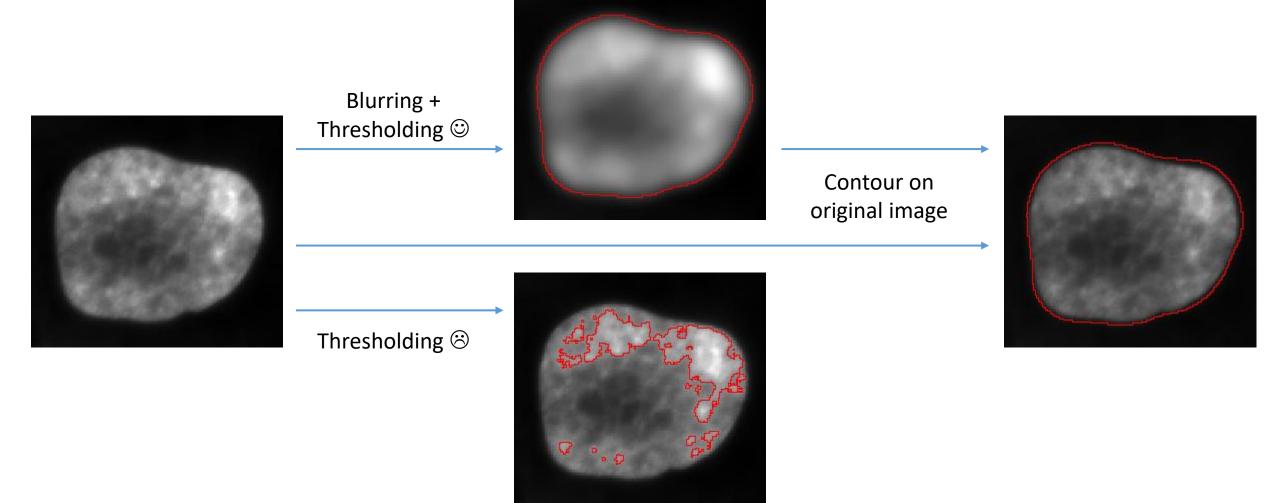


- Gaussian filter
- Median filter (computationally expensive)



# Filtering for improving thresholding results

- In case thresholding algorithms outline the wrong structure, <u>blurring in advance</u> may help.
- However: **Do not** continue processing the blurred image, continue with the original!

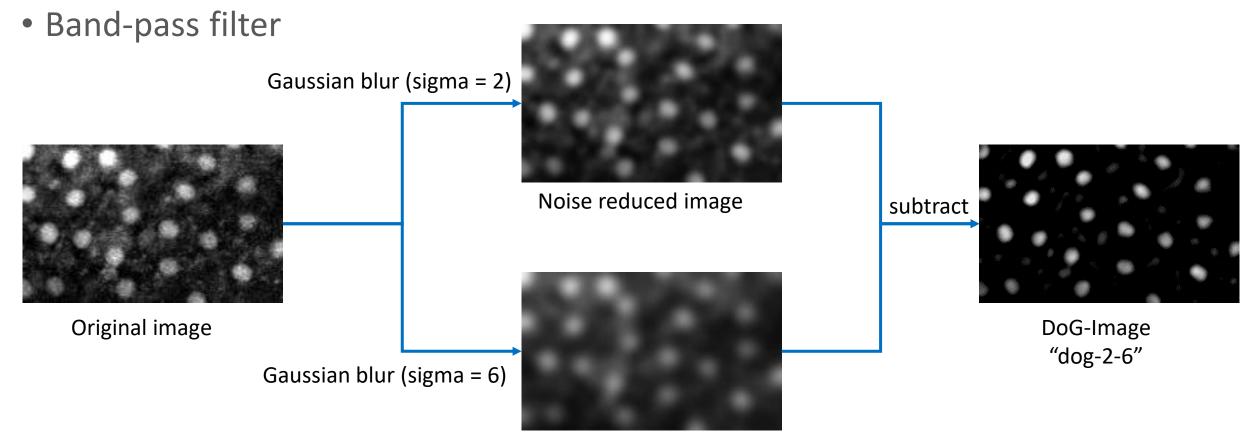








• Improve image in order to detect bright objects.



Background image



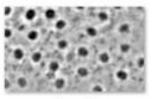
#### Difference-of-Gaussian (DoG)

PoL Physics of Life **TU Dresden** 

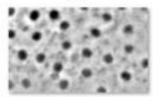
Example DoG images



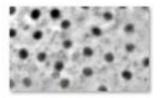
dog-1-1



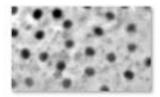
dog-4-1



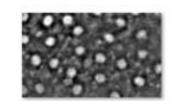
dog-7-1



dog-10-1



dog-13-1



dog-1-4



dog-4-4



dog-7-4



dog-10-4



dog-13-4



dog-1-7



dog-4-7



dog-7-7



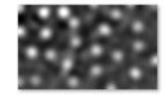
dog-10-7



dog-13-7



dog-1-10



dog-4-10



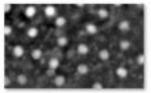
dog-7-10



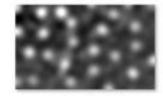
dog-10-10



dog-13-10



dog-1-13



dog-4-13



dog-7-13



dog-10-13



dog-13-13

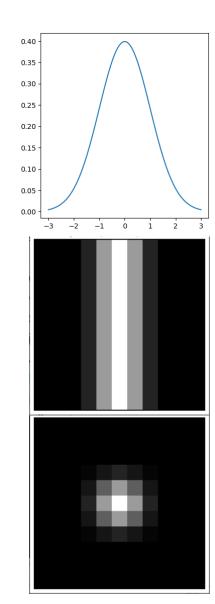


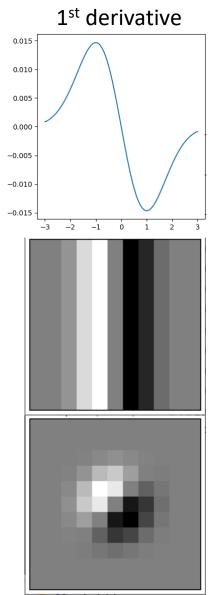


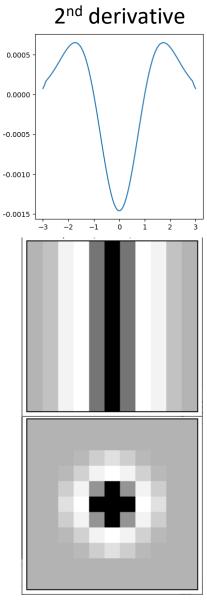
# Laplace-filter



- Second derivative of a Gaussian blur filter
- Used for edge-detection and edge enhancement
- Also known as the *Mexican-hat-filter*

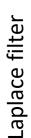


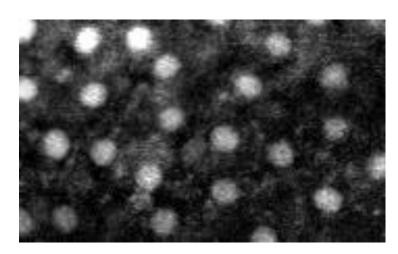






#### Laplacian-of-Gaussian (LoG)

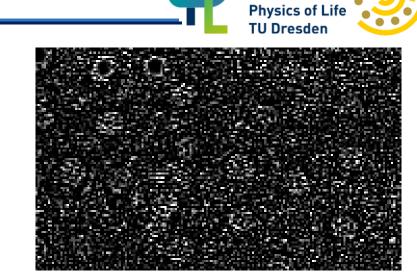






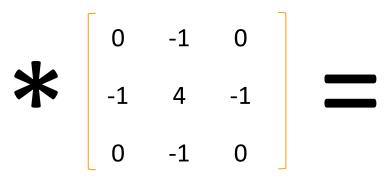


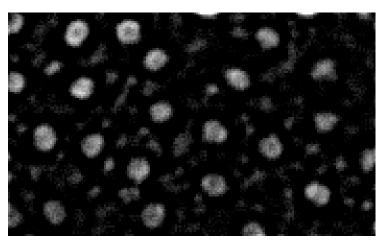
0 0 -1 0 0



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Laplace filtered image



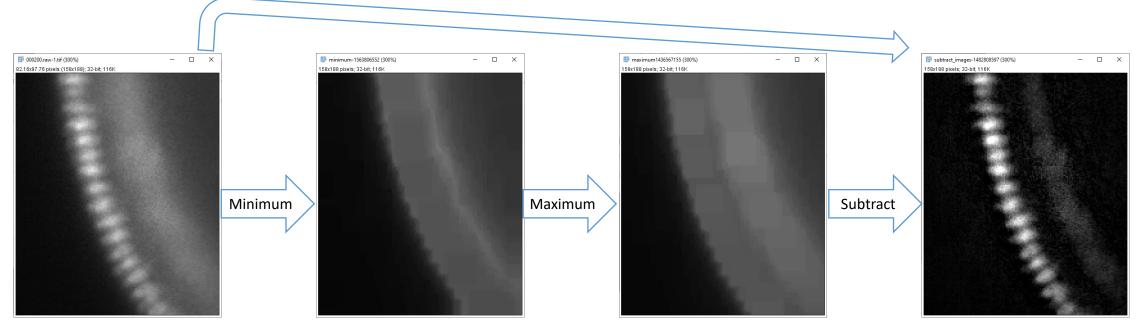


LoG image

# Top-hat filter



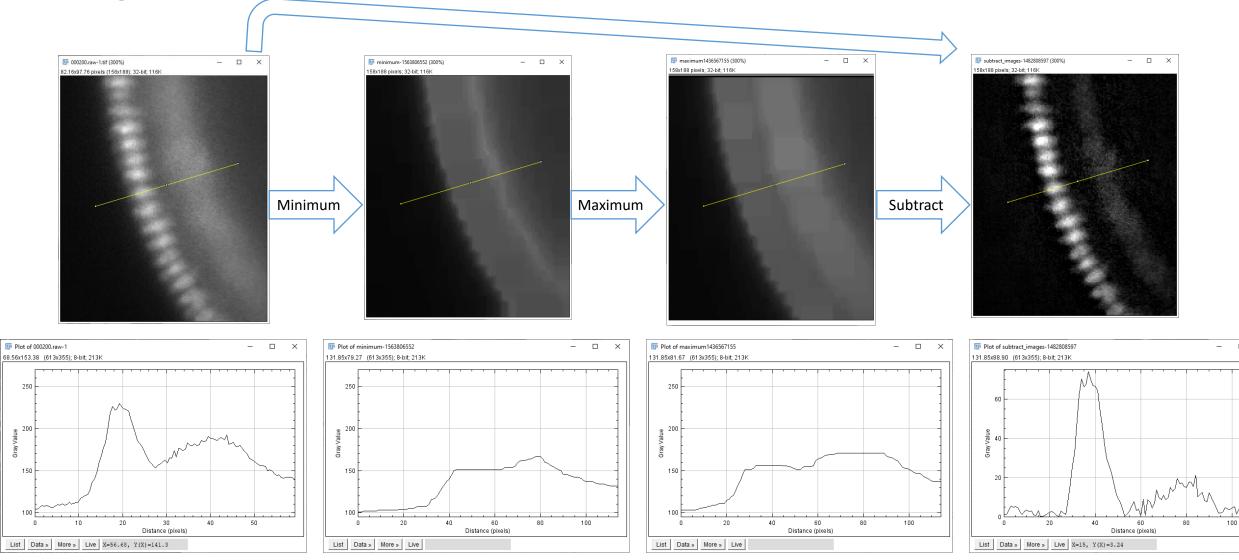
• Background subtraction



### Top-hat filter



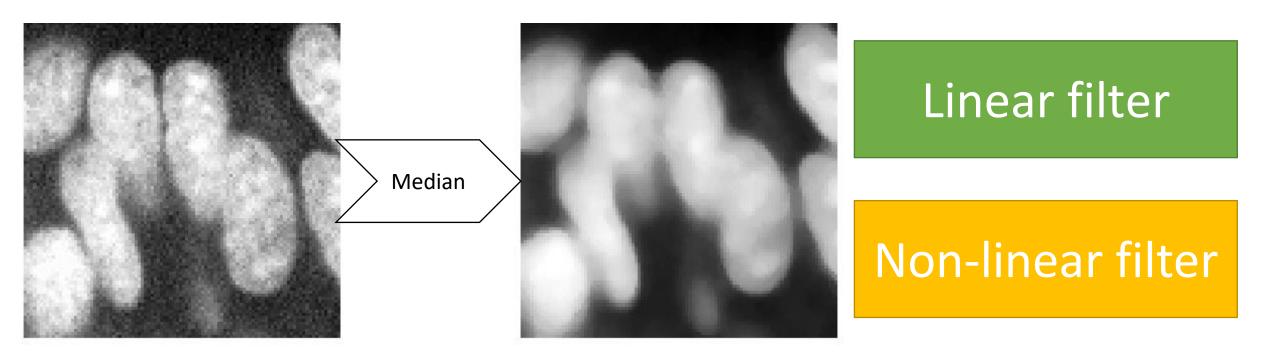
• Background subtraction



🥑 @haesleinhuepf

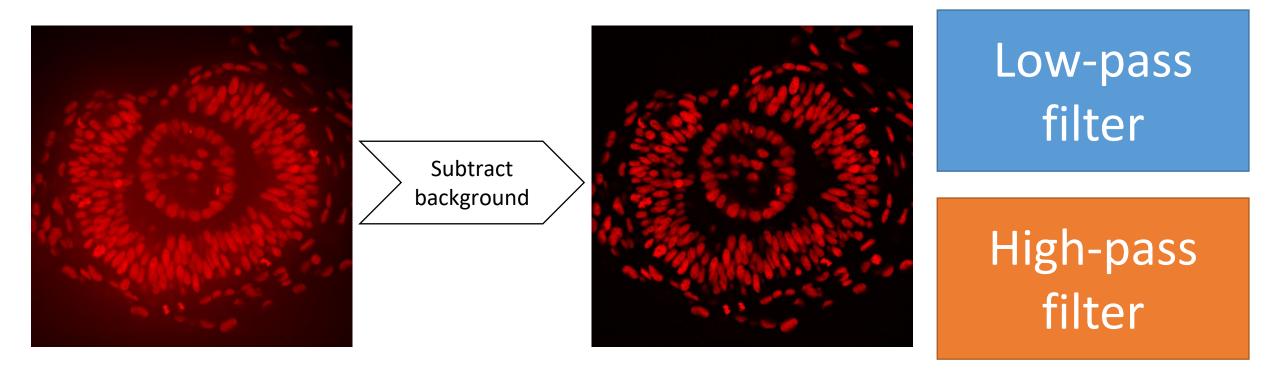


### • The median filter is a ...





# • Removing background from an image is a ... ?







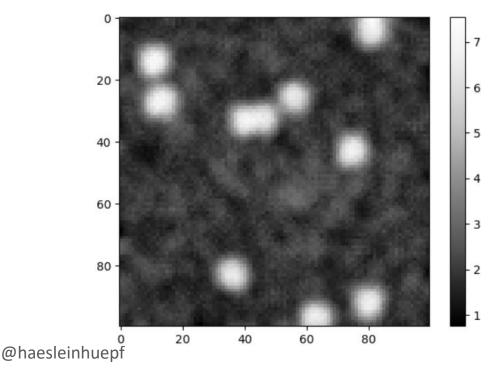
#### Short detour: Image segmentation Robert Haase

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Thresholding

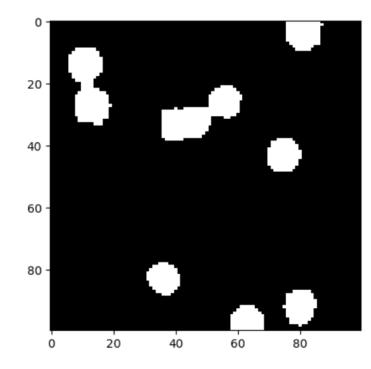
- Very basic and yet efficient segmentation technique
- Histogram based, to determine an intensity threshold
- Not state-of-the-art in many fields (anymore)

# Intensity image



# **Binary image**

TU Dresde





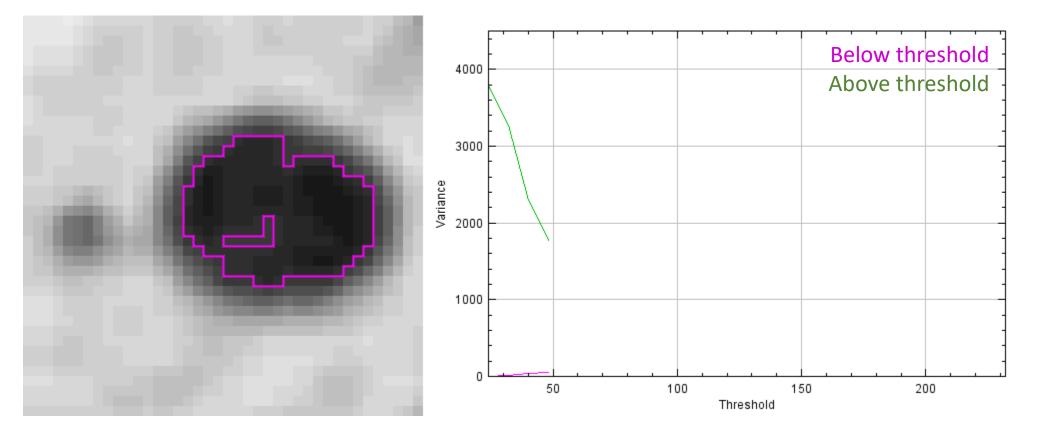
 $Var(I) = \sum_{i \in I} g_i - \bar{g}_I$ 

$$\bar{g}_I = \sum_{i \in I} \frac{g_i}{n_I}$$

Var(I) ... Variance in image I  $g_i$  ... grey value of a pixel i  $\overline{g}_I$  ... mean grey value of the whole image I  $n_I$  ... number of pixels in Image I

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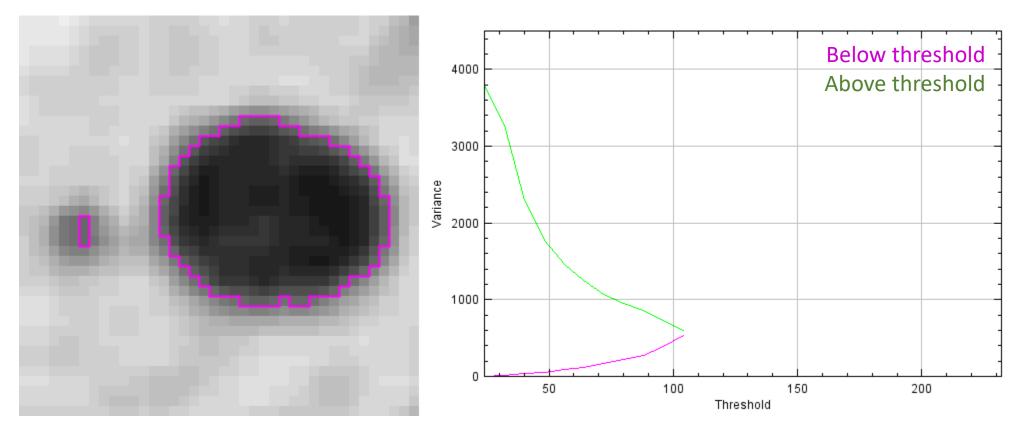
 $Var(I) = \sum_{i \in I} g_i - \bar{g}_I$ 

$$\bar{g}_I = \sum_{i \in I} \frac{g_i}{n_I}$$

Var(I) ... Variance in image I  $g_i$  ... grey value of a pixel i  $\overline{g}_I$  ... mean grey value of the whole image I  $n_I$  ... number of pixels in Image I

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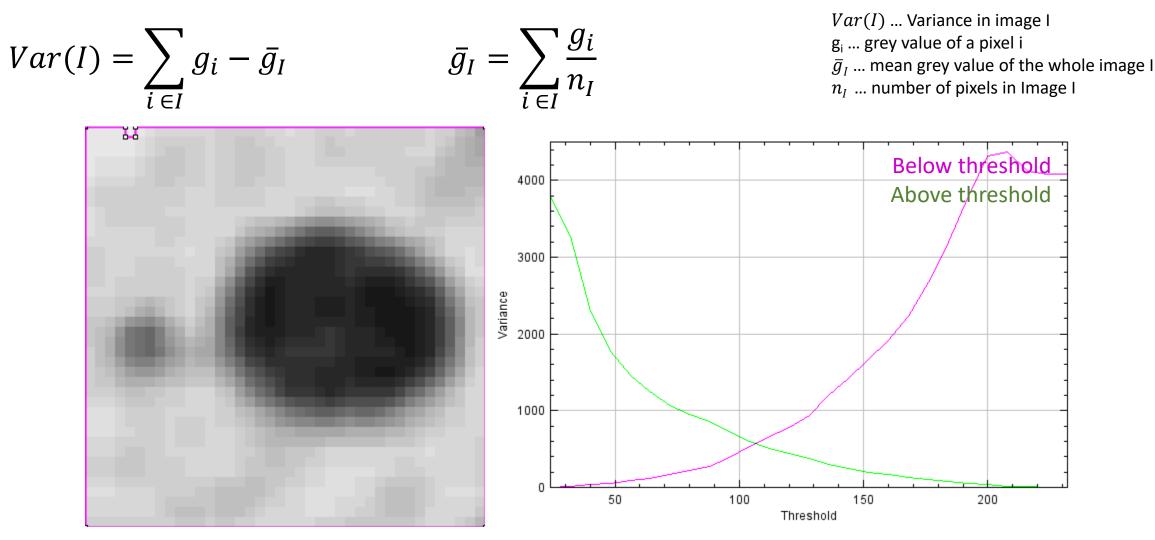






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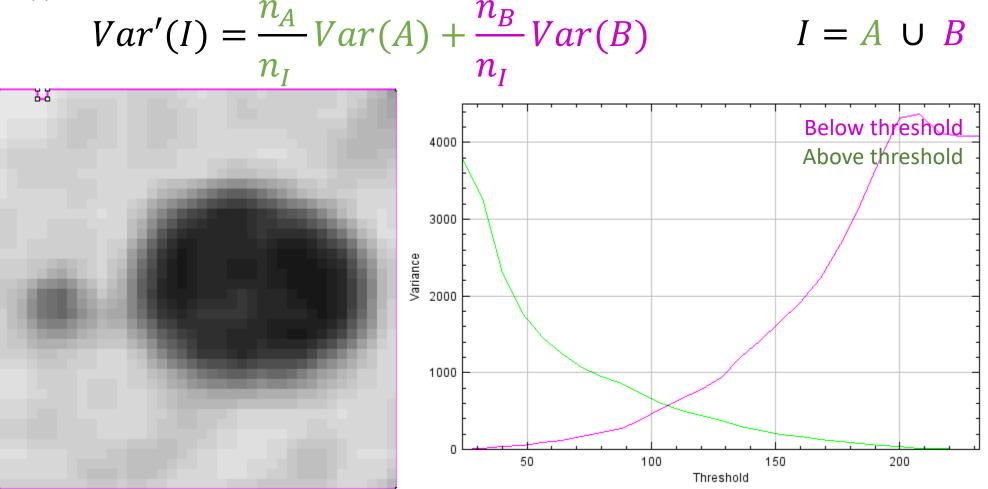
🍯 @haesleinhuepf

• Searching for a threshold where the variance in both classes (above/below threshold) becomes minimal.

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• Weighted (!) sum variance

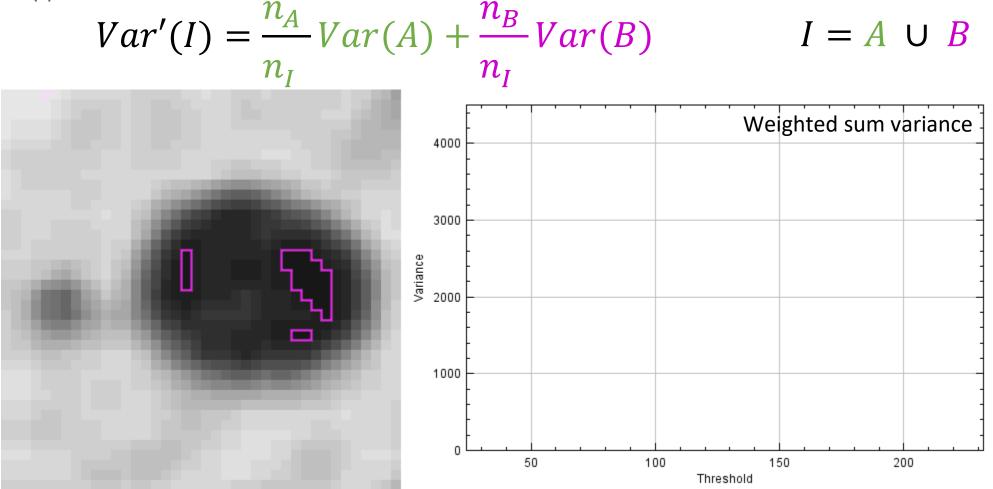




• Searching for a threshold where the variance in both classes (above/below threshold) becomes minimal.

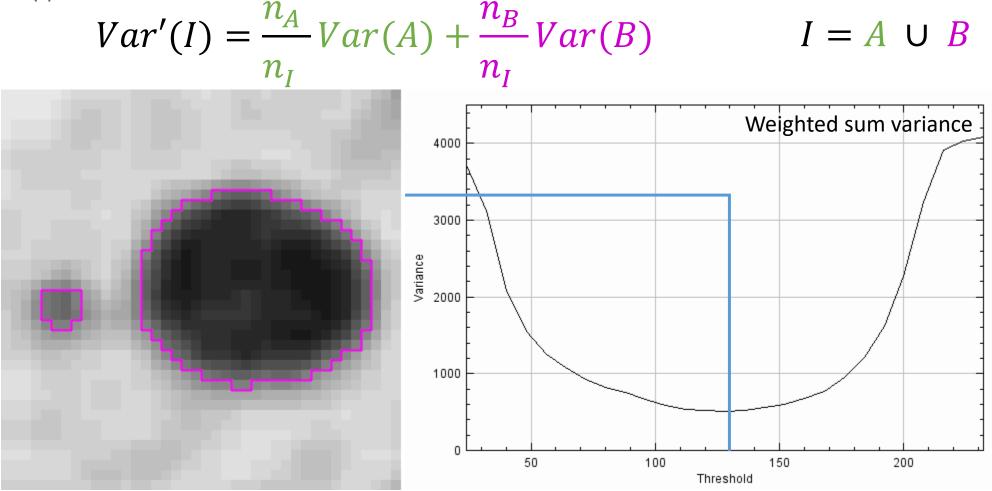
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• Weighted (!) sum variance





- Searching for a threshold where the variance in both classes (above/below threshold) becomes minimal.
- Weighted (!) sum variance



See also: http://www.labbookpages.co.uk/software/imgProc/otsuThreshold.html

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• Cite the thresholding method of your choice properly

"We segmented the cell nuclei in the images using Otsu's thresholding method (Otsu et Al. 1979) implemented in scikit-image (van der Walt et al. 2014)."

#### IEEE TRANSACTIONS ON SYSTEMS, MAN, AND CYBERNETICS, VOL. SMC-9, NO. 1, JANUARY 1979

#### A Threshold Selection Method from Gray-Level Histograms

#### NOBUYUKI OTSU

Abstract—A nonparametric and unsupervised method of automatic threshold selection for picture segmentation is presented. An optimal threshold is selected by the discriminant criterion, namely, so as to maximize the separability of the resultant classes in gray levels. The procedure is very simple, utilizing only the zeroth- and the first-order cumulative moments of the gray-level histogram. It is straightforward to extend the method to multithreshold problems. Several experimental results are also presented to support the validity of the method.







## Image Processing: Morphological Operations Robert Haase

#### With material from

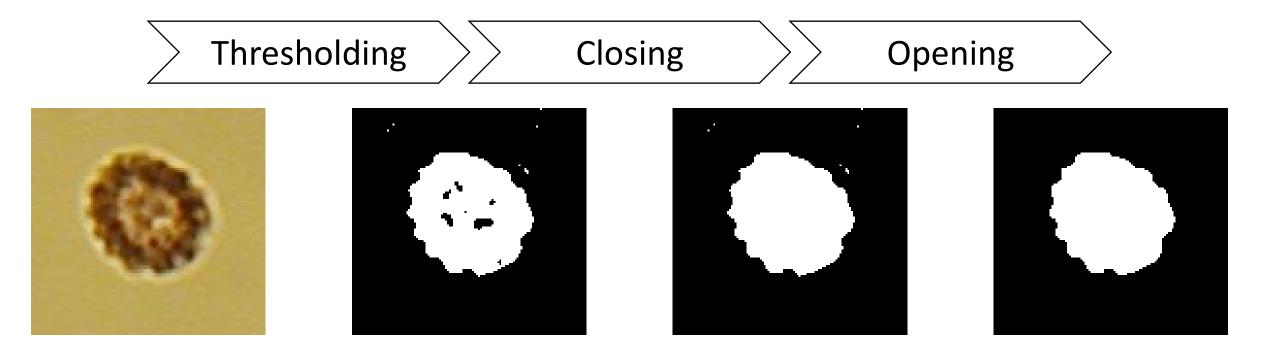
Marcelo Leomil Zoccoler, Physic of Life, TU Dresden

🈏 @haesleinhuepf

### Refining masks



- Binary mask images may not be perfect immediately after thresholding.
- There are ways of refining them

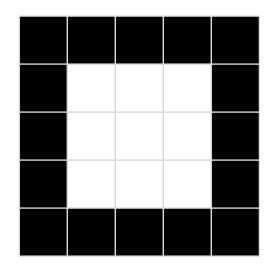


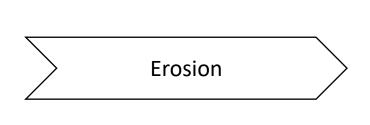






• Erosion: Every pixel with at least one black neighbor becomes black.



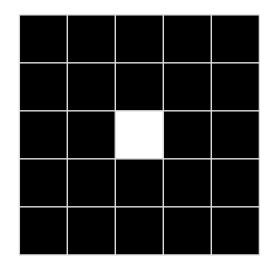


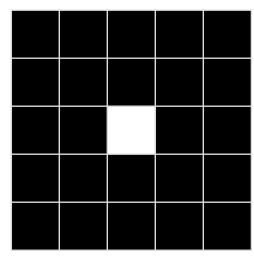


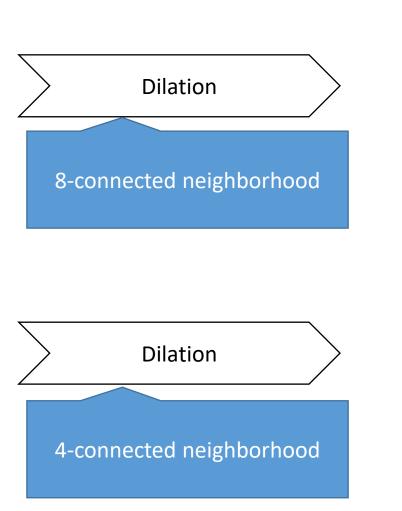
#### Dilation

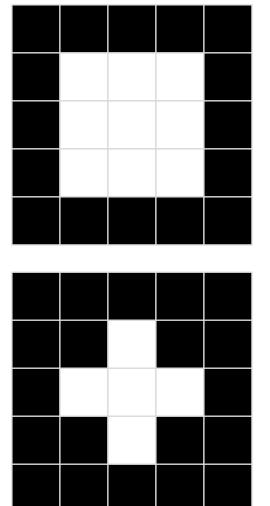


• Dilation: Every pixel with at least one white neighbor becomes white.







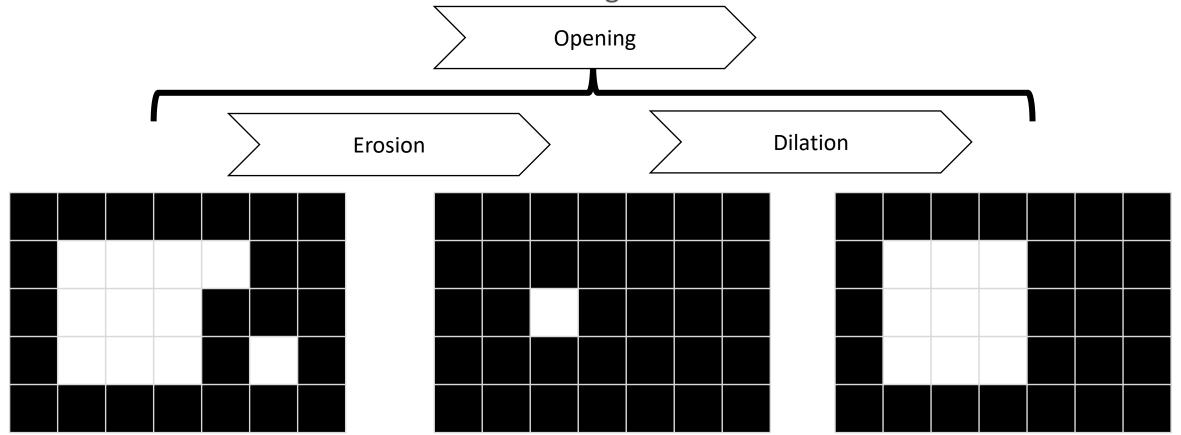


) @haesleinhuepf

## Opening



• Erosion and dilation combined allow correcting outlines.

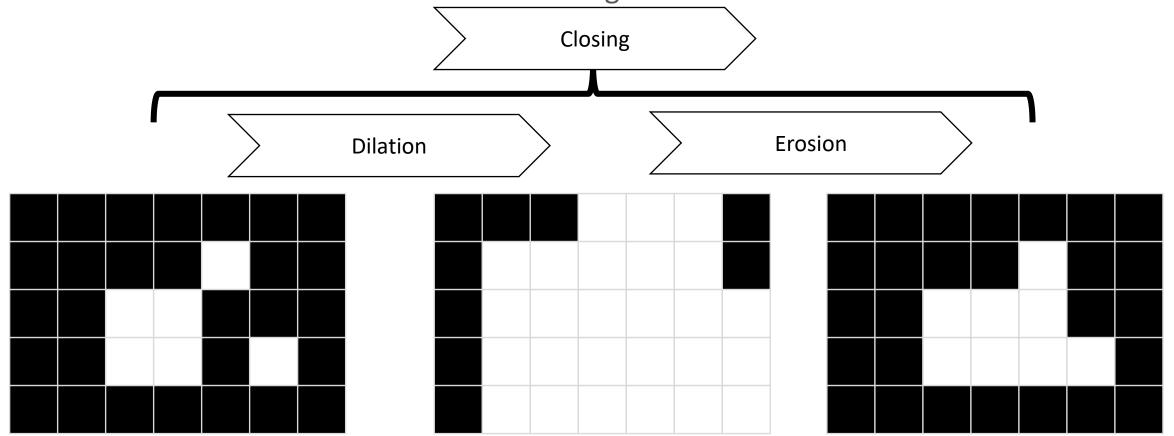


- It can separate white (high intensity) structures that are weakly connected
- It may erase small white structures

## Closing

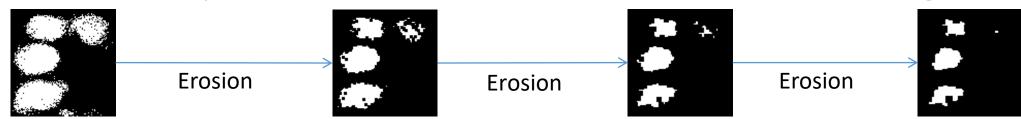


• Erosion and dilation combined allow correcting outlines.



- It can connect white (high intensity) structures that are nearby
- It may close small holes inside structures

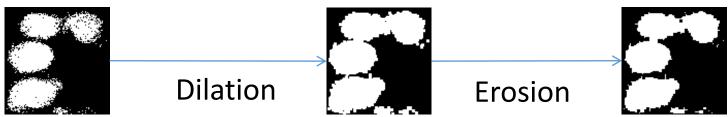




• Dilation: Set all pixels to white which have at least one white neighbor.



• Closing: Dilation + Erosion



• Opening: Erosion + Dilation





# Image Processing in Python

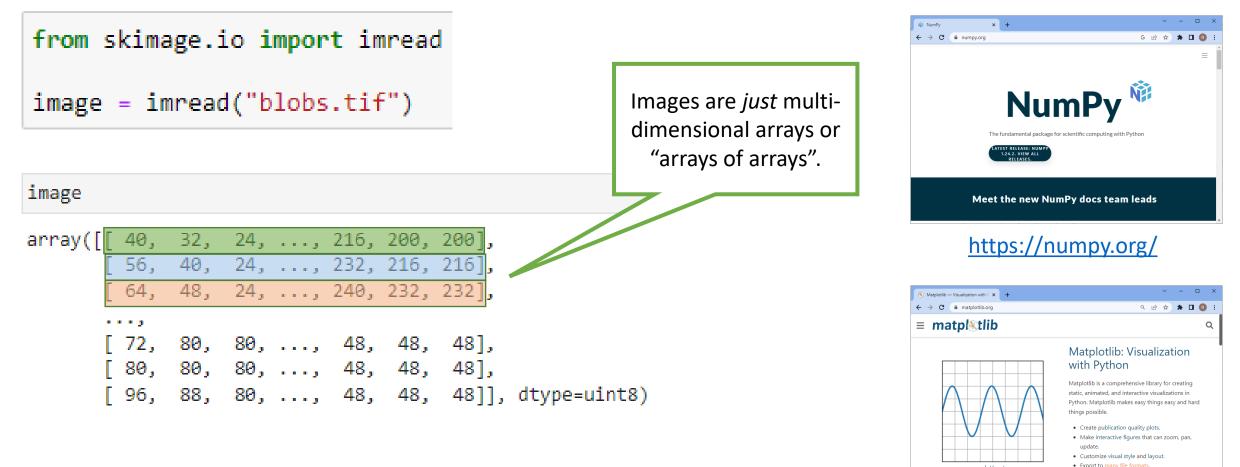
**Robert Haase** 

With material from

Marcelo Leomil Zoccoler, Physics of Life, TU Dresden

Pol Physics of Life TU Dresden

• Open images



https://matplotlib.org/

plot(x, y)

### Working with images in python



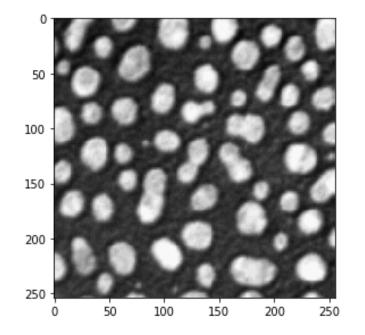
• Open images

from	skimage.io	import	imread
image	e = imread('	'blobs.t	if")

#### Visualize images

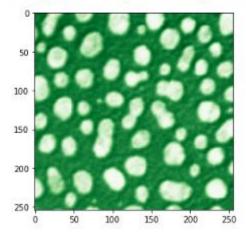
from skimage.io import imshow imshow(image)

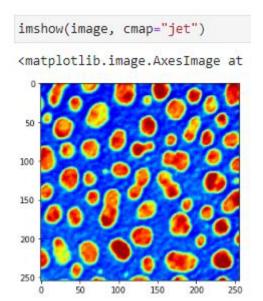
<matplotlib.image.AxesImage at 0x245e7(



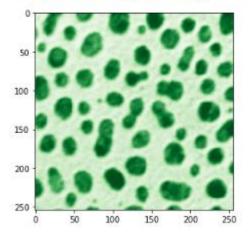
imshow(image, cmap="Greens\_r")

<matplotlib.image.AxesImage at 0: <matplotlib.image.AxesImage at 0</pre>





imshow(image, cmap="Greens")



This does not modify the image data. The images are just shown with different colors representing the same values.

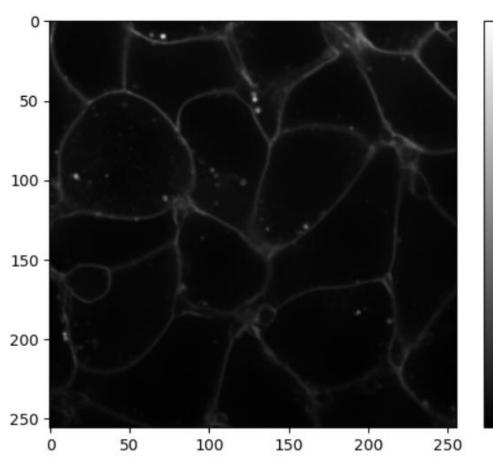
@haesleinhuepf

#### Brightness, contrast, display-range



• After loading data, make sure you can see the structure you're interested in

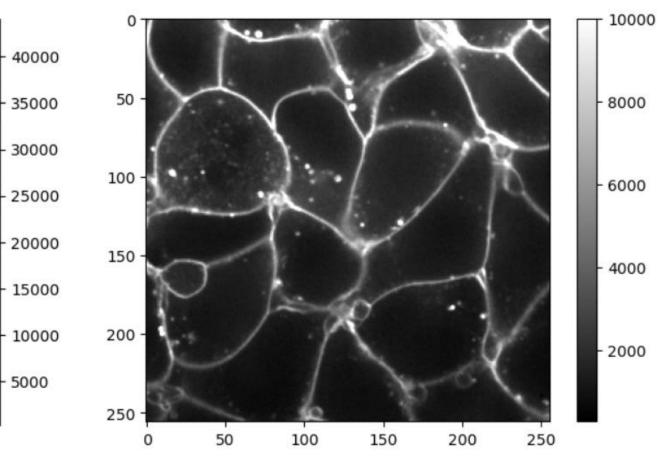
```
plt.imshow(image, cmap='gray')
plt.colorbar()
```



<matplotlib.colorbar.Colorbar at 0x14f22cf71f0>

plt.imshow(image, cmap='gray', vmax=10000)
plt.colorbar()

<matplotlib.colorbar.Colorbar at 0x14f22d70310>



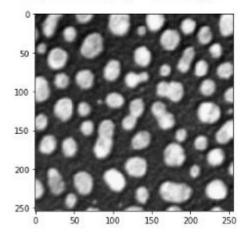
🍯 @haesleinhuepf

## Cropping and resampling images

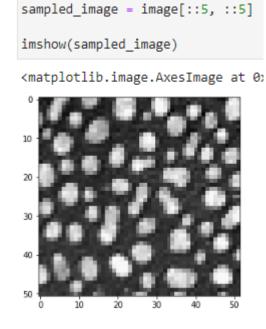
• Indexing and cropping *numpy-arrays* works like with python arrays.

#### imshow(image)

<matplotlib.image.AxesImage at 6



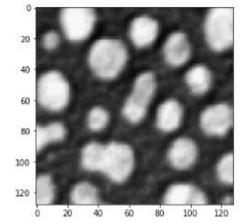
#### Original image



cropped\_image2 = image[0:128, 128:]

imshow(cropped\_image2)

<matplotlib.image.AxesImage at 0x29e

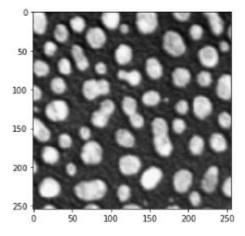


Cropped image

flipped\_image = image[::, ::-1]
imshow(flipped\_image)

<matplotlib.image.AxesImage at 0x

Physics of Life TU Dresden



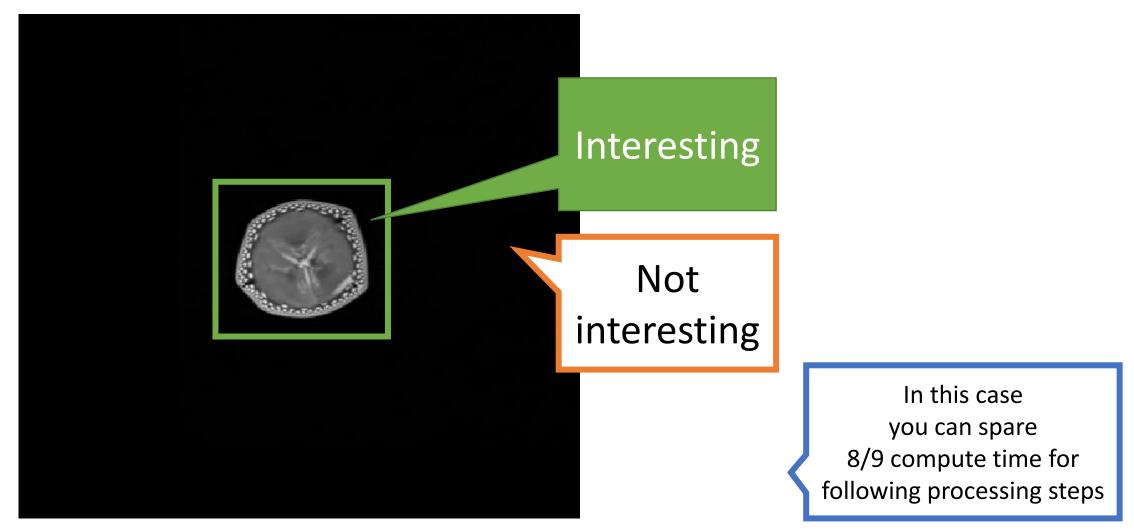
Flipped image

#### Sub-sampled image





• Crop out the region you're interested in





#### Filters

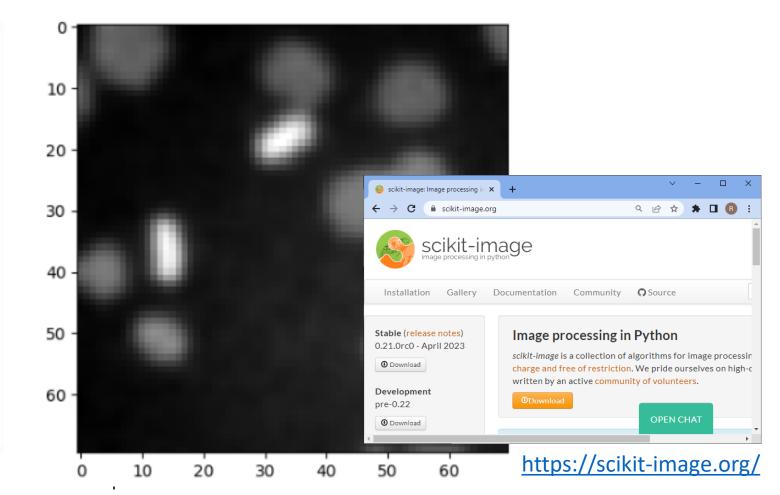


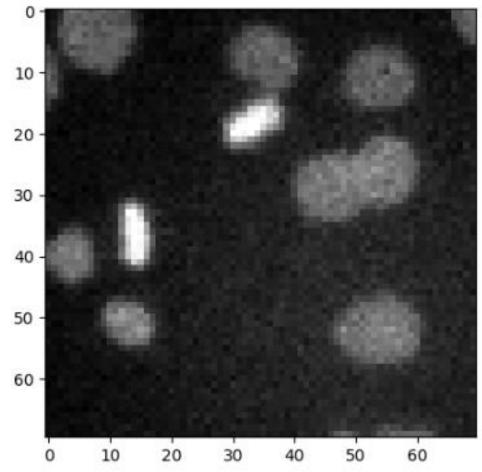


denoised\_gaussian = filters.gaussian(image3, sigma=1)

plt.imshow(denoised\_gaussian, cmap='gray')

<matplotlib.image.AxesImage at 0x283aab3ba90>





Filters

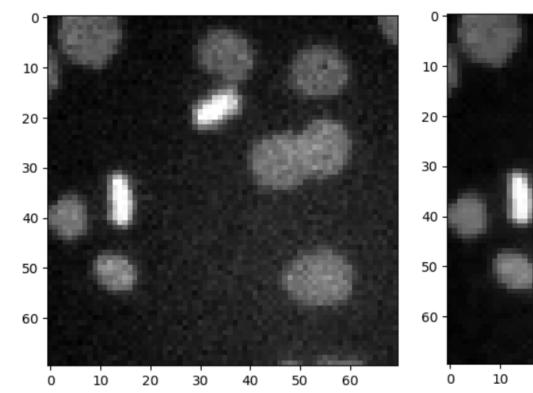


 Use every opportunity and play with filter parameters to get an idea what they do.

denoised\_median = filters.median(image3, morphology.disk(1))

plt.imshow(denoised median, cmap='gray')

plt.imshow(image3, cmap='gray')

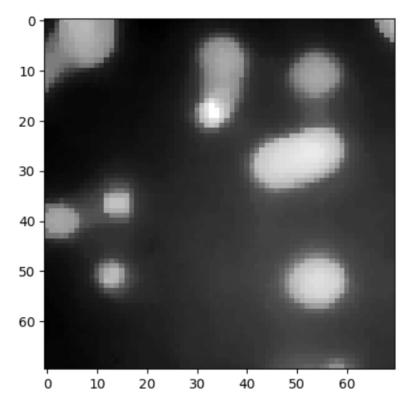


<matplotlib.image.AxesImage at 0x1d86893b6d0>

denoised\_median2 = filters.median(image3, morphology.disk(5))

plt.imshow(denoised\_median2, cmap='gray')

<matplotlib.image.AxesImage at 0x1d868ca7af0>



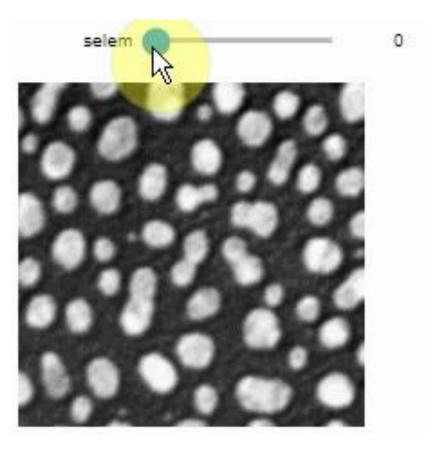
🥑 @haesleinhuepf





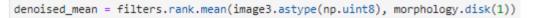
 Use every opportunity and play with filter parameters to get an idea what they do.

from skimage.filters.rank import maximum
stackview.interact(maximum, slice\_image)



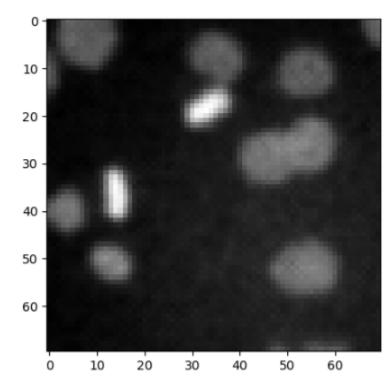


#### ... are just functions

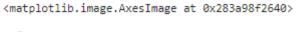


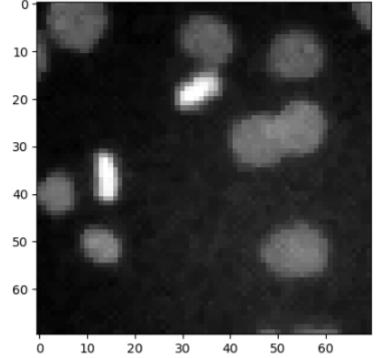
plt.imshow(denoised\_mean, cmap='gray')

<matplotlib.image.AxesImage at 0x283a9868310>



denoised\_median = filters.median(image3, morphology.disk(1))
plt.imshow(denoised\_median, cmap='gray')

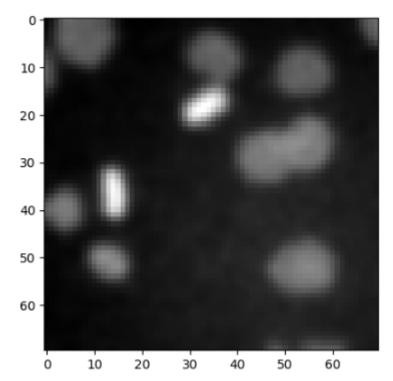




denoised\_gaussian = filters.gaussian(image3, sigma=1)

plt.imshow(denoised\_gaussian, cmap='gray')

<matplotlib.image.AxesImage at 0x283aab3ba90>







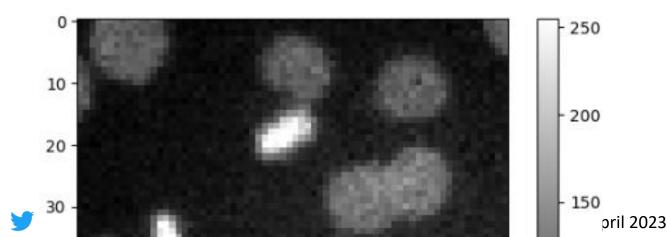
... may be custom functions

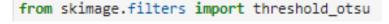
Recommendation: Apply custom filters to super simple images to see if they do the right thing.



## **Binarization / Thresholding**

- Turn images into binary images (very basic form of segmentation)
- When using scikit-image, threshold\_functions typically return a threshold you need to apply yourself.



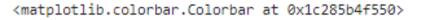


```
threshold = threshold_otsu(image_nuclei)
threshold
```

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image\_otsu\_binary = image\_nuclei > threshold

```
plt.imshow(image_otsu_binary, cmap='gray')
plt.colorbar()
```

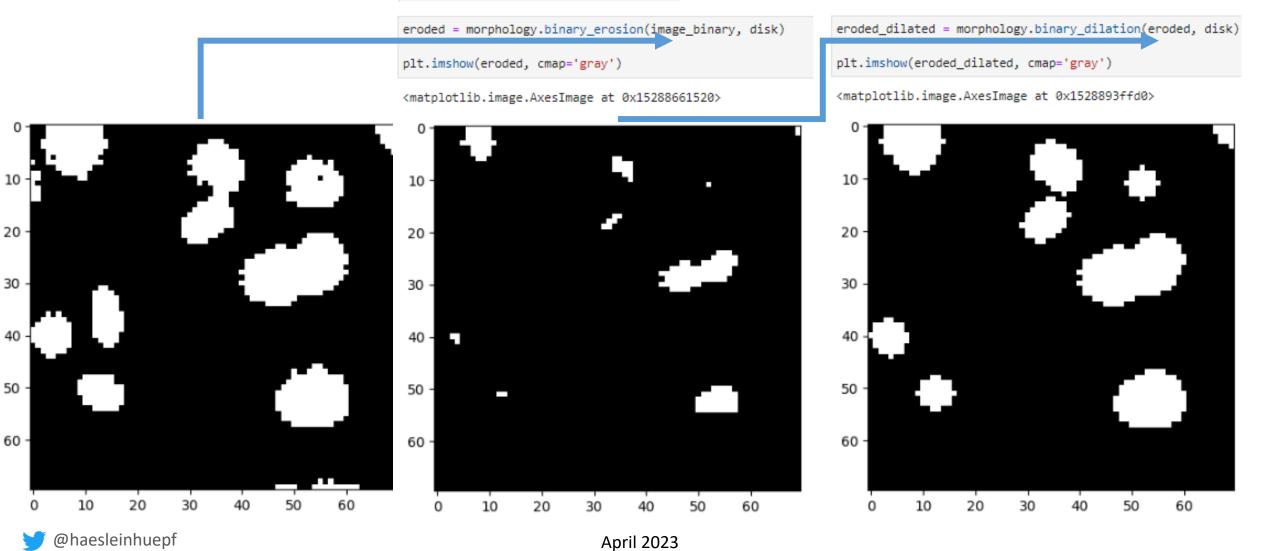






• To *morph* objects in binary images

#### from skimage import morphology







# Image visualization in Python using Napari

**Robert Haase** 

With material from

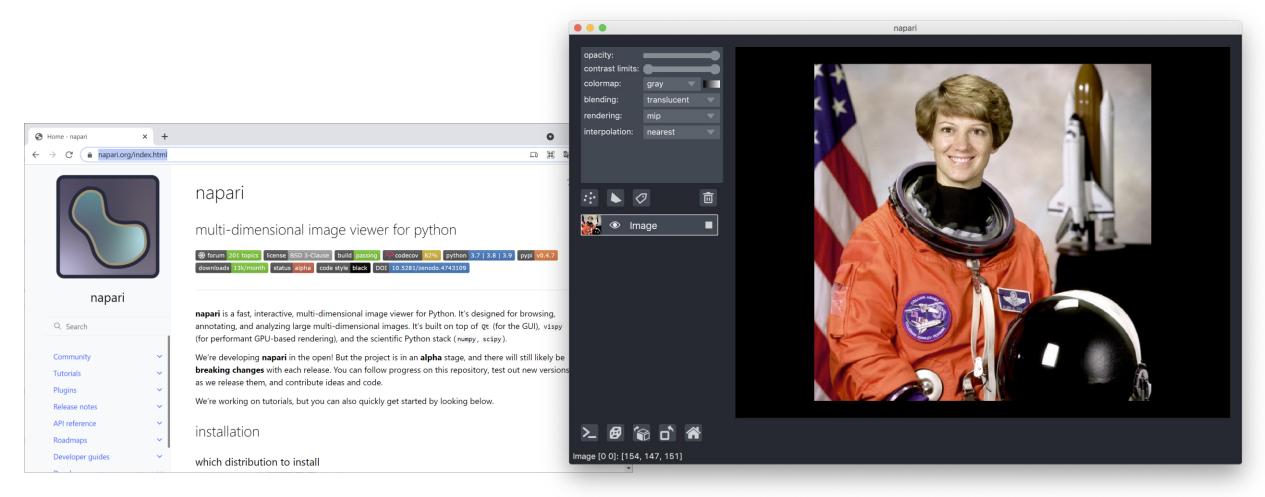
Marcelo Leomil Zoccoler, Physics of Life, TU Dresden April 2023



# Napari: 3D viewer for Python

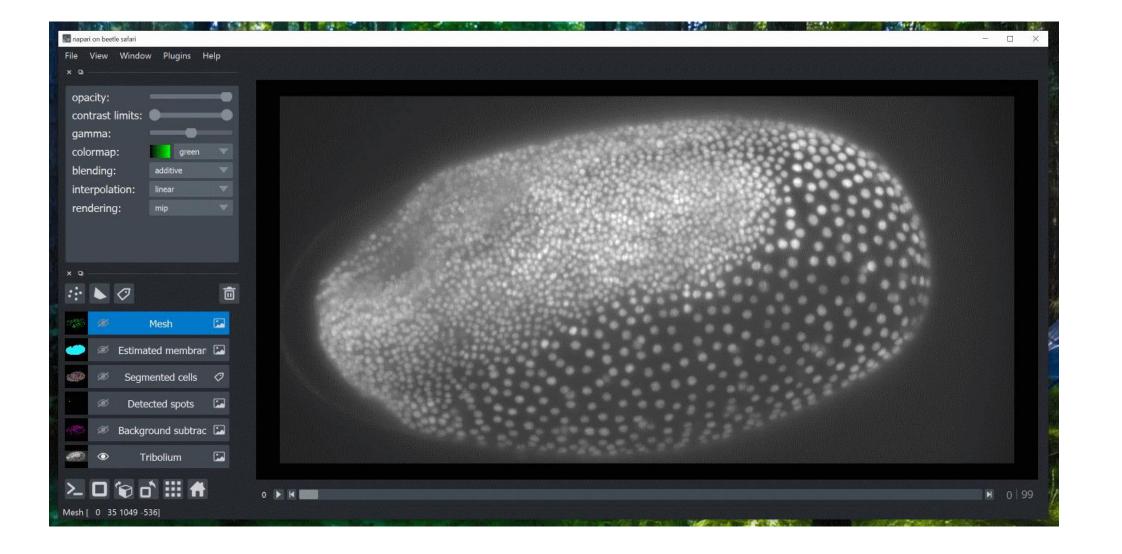


• Multi-dimensional image viewer in Python



https://napari.org/

## Napari: 3D viewer for Python

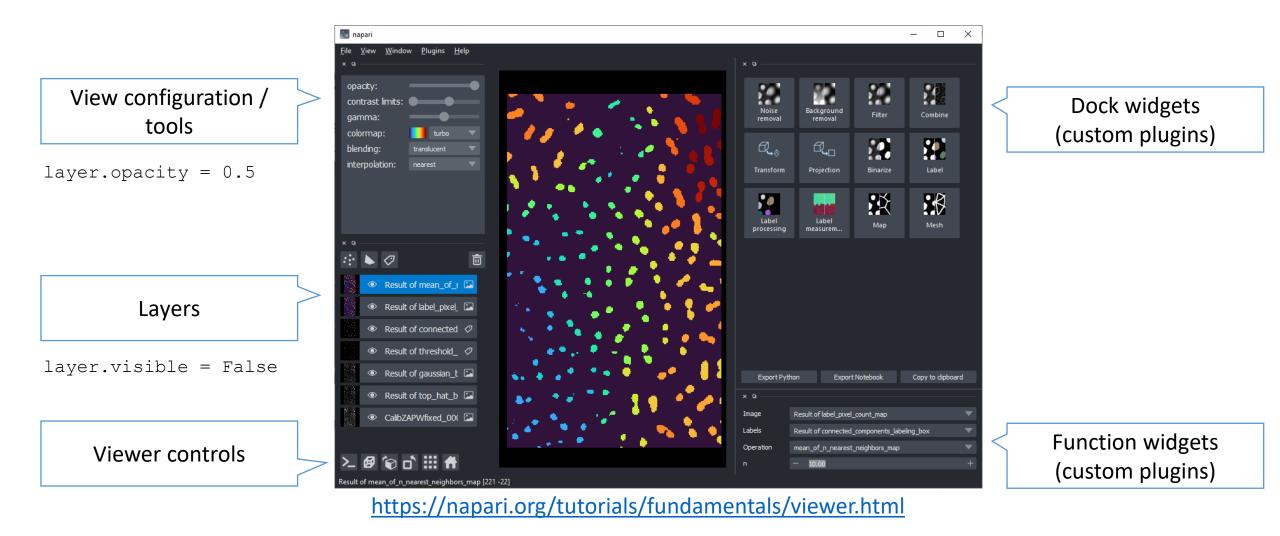


PoL Physics of Life

**TU Dresden** 

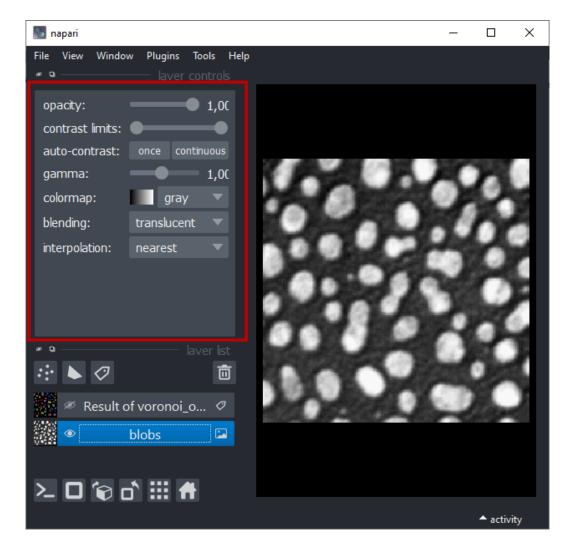


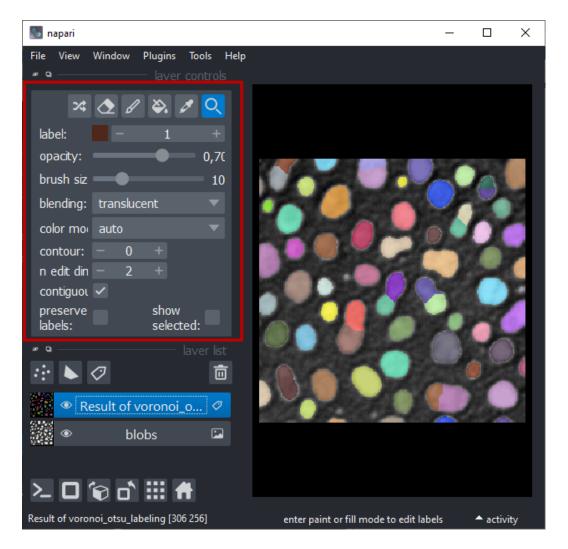






• Different layers have different configurations



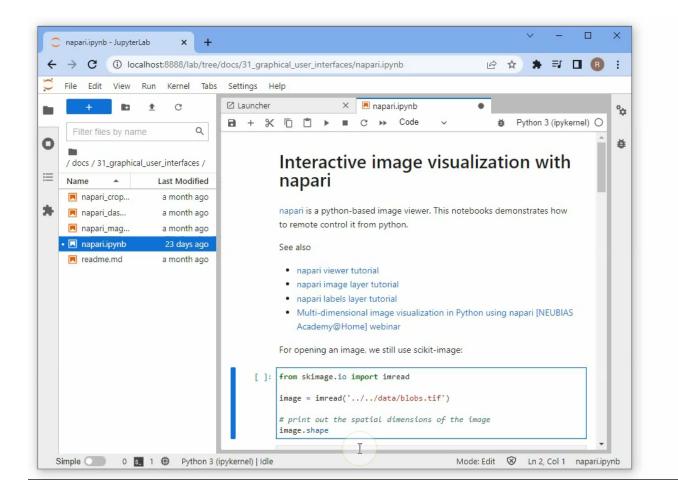


🥑 @haesleinhuepf

#### Using Napari from Python Code



• A great mix of interactivity and reproducibility



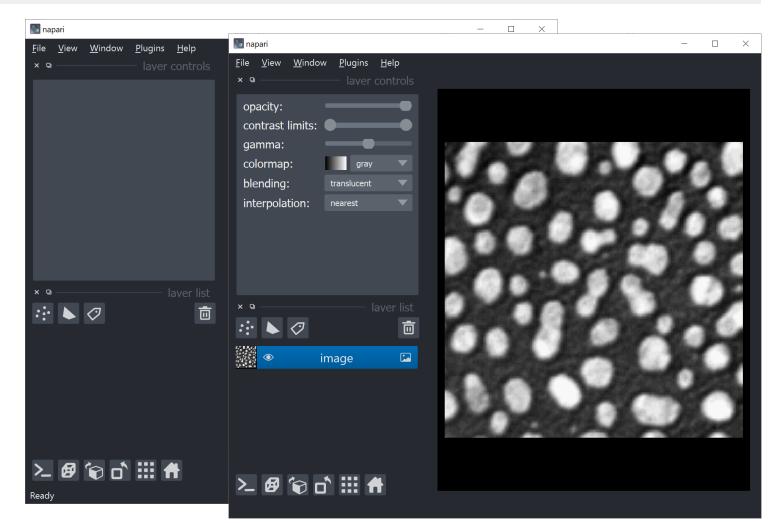
# Scripting napari

Initialization
 import napari

# Create an empty viewer
viewer = napari.Viewer()

• Adding images

viewer.add\_image(image)

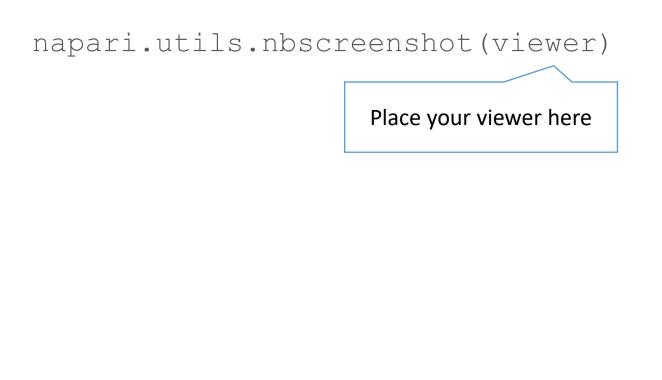




# Scripting napari in notebooks



• Make screenshots from napari and put them in your jupter notebook



	01_napari - Jupyter Notebook × +	- 🗆	×
$\leftarrow$	→ C ③ localhost:8889/notebooks/01_napari.jpynb	☆ R	:
	Cjupyter 01_napari (autosaved)	Logout	
	File Edit View Insert Cell Kernel Widgets Help Not Trusted	Python 3 O	
	<pre>In [1]:  import napari # Create an empty viewer viewer = napari.Viewer() # Start it napari.run() In [3]:  # Add a new Layer containing an image viewer.add_image(image) Out[3]: <image 'image'="" 0x1a72ea05580="" at="" layer=""/> With this command, we can make a screenshot of napari and save it in our notebook.</pre>		
	<pre>In [6]: M napari.utils.nbscreenshot(viewer) Out[6]: File View Window Plugins Help opacity: contrast lmits: gamma: colormap: gray blending: translucent interpolation: nearest</pre>	W	

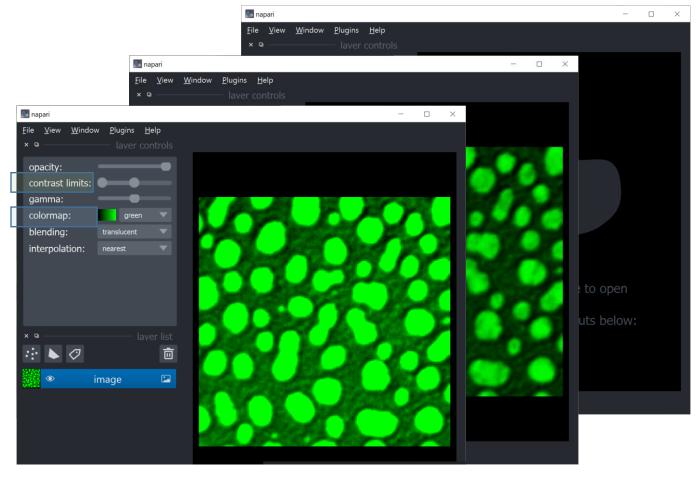




) @haesleinhuepf

# Working with layers

- Removing layers
   for l in viewer.layers:
   viewer.layers.remove(l)
- Modify visualization while adding layers viewer.add\_image(image, colormap='green')
- Modify layers after adding
- layer = viewer.add\_image(image)
  layer.colormap = 'green'
  layer.contrast\_limits = (0, 128)





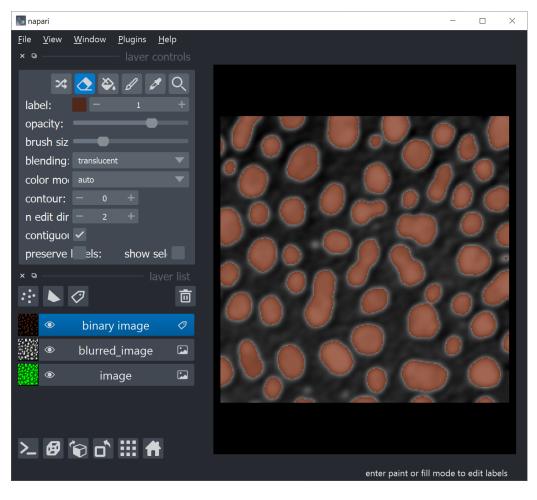


# Visualizing image segmentation

• Binary images and label images visualized as label layers

```
from skimage.filters import threshold_otsu
threshold = threshold_otsu(blurred_image)
binary image = blurred image > threshold
```

```
# Add a new labels layer containing an image
viewer.add_labels(binary_image)
```







#### Summary



- Image visualization
  - Pixel size, colormaps, bit-depth
  - Image histogram
  - Brightness/Contrast
- Image Filtering
- Morphological Operations
  - Mask Refinement

- Python libraries
  - Matplotlib
  - Scikit-image
  - Napari

#### Coming up next

- Image Segmentation
  - Connected component analysis
  - Voronoi-Otsu-Labeling
- Surface reconstruction

